cannot afford to have treatment failures due to inadequate dosing. Further, the high dose tablets will reduce the loads carried and the cost for the same dose of active ingredient. The cost of 20 tablets of the various drugs mentioned varies from £1.80 to £9.00 (BNF) but many pharmaceutical companies may be willing to donate supplies for a reduced price or even to provide them free of charge. Intravenous metronidazole is particularly expensive and on a dose for dose basis is approximately ten times the cost of the rectal preparation which is equally efficacious under most circumstances.

All antibiotics should be kept dry and as cool as possible. It is suggested that they should all be kept at less than 25°C but this may not always be possible.

The final list of recommended antibiotics comprises:

- Injectable cefuroxime
- Oral trimethoprim (or co-trimoxazole)
- Oral erythromycin
- Oral flucloxacillin
- Rectal metronidazole
- Appropriate anti-malarial compound for treatment
- Acyclovir cream
- Lindane preparation

The quantity of each drug taken will depend on the size and duration of the expedition and also the area to be visited. Obviously these antibiotics for which more than one indication has been discussed above should be taken in greater quantity. If it is practicable to take a second parenteral preparation then trimethoprim is recommended.

In many ways, the ideal 'expedition antibiotic' would be chloramphenicol. It is available as an oral or injectable compound and is highly effective in nearly all of the clinical circumstances discussed. It is also relatively inexpensive. Unfortunately its potential side-effects make it an unacceptable choice. Perhaps in the future, one of the quinolones may be the one drug to take.

ALASTAIR R. O. MILLER
Department of Communicable and Tropical Diseases, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST, England

References


Newer mechanisms of resistance to \( \beta \)-lactam antibiotics in Gram-negative bacteria

The cell envelope provides a barrier between the bacterial cell and its environment, with the capsule or exopolysaccharide providing the primary barrier and therefore the initial obstacle in the penetration of \( \beta \)-lactam antibiotics to their targets, the penicillin-binding proteins (PBPs). There are three ways in which this first layer can act as a permeability barrier to antibiotics (Slack & Nichols, 1982), firstly the capsule or exopolysaccharide could form a static layer through which the antibiotic must diffuse to enter the bacterial cell envelope, the deeper the layer the longer the time the molecule will take to diffuse. The second mechanism would be the frictional resistance to diffusion afforded by the structural matrix of the polysaccharides. The third hypothetical mechanism would be a reduction in permeation of a charged antibiotic through an oppositely charged matrix (Helfferich, 1962). Stanier, Adelberg & Ingraham (1977) showed that the capsular materials and exopolysaccharides of clinically important bacteria were commonly anionic or less frequently neutral, therefore a positively charged \( \beta \)-lactam antibiotics would have to saturate any free binding sites in the matrix before penetrating to the bacterial surface. Reduced penetration of a positively charged aminoglycoside has been demonstrated in vitro with hydrated exopolysaccharide from Pseudomonas aeruginosa (Slack & Nichols, 1981), this effect was not seen with neutral or negatively charged \( \beta \)-lactams.

Once the antibiotic has penetrated the external polysaccharides (if present) the outer membrane provides the next barrier. It is
known that the outer membranes from Gram-negative rods are resistant to hydrophobic antibiotics such as fusidic acid and rifamycin but vary in their susceptibility to hydrophilic compounds (Chopra & Howe, 1978). There is evidence that one of the mechanisms of β-lactams entering the cell is via the porin channels (Nikaido, Rosenberg & Foulds, 1983) and that modifications to the proteins which form these channels can cause a decrease in susceptibility to β-lactam antibiotics. It is thought that hydrophobicity and size of the antibiotic are important in deciding the ease with which the compound can pass through the porin channels. Some antibiotics may encounter difficulty in traversing the outer membranes if they are lipid soluble (i.e. hydrophobic) or larger than 600 daltons or both and therefore be intrinsically less effective antibiotics (Nikaido et al., 1983). Jaffe, Chabbert & Semolin (1982) compared isogenic strains of E. coli K-12 lacking the porin proteins OmpF or OmpC or both for resistance to cefoxitin, ampicillin, cephaloridine and cefazolin, it was concluded that both proteins form channels for these antibiotics but that OmpF was more efficient, and that in double mutants (ompF- ompC-) there was decreased susceptibility to first and second generation cephalosporins. Harder, Nikaido & Matsuhashi (1981), showed that resistance in E. coli to carbenicillin was concomitant with the loss of ompF porin. However, there must be another (so far undetected) route through the outer membrane for β-lactams as ampicillin has been shown to permeate the outer membrane easily in porin defective mutants; this route is not via the Lam B protein or the Tsp protein (Sawai et al., 1982). There was evidence that there was significant passage of ampicillin (but not cephalosporins) through the phospholipid. Evidence of porins not being the only route of entry for β-lactams was also shown by Curtis et al., (1985) where E. coli mutants losing ompF and ompC showed no change in susceptibility to aztreonam or the aminothiazoyl cephalosporins (ceftotaxime, cefetazidine, cefetimoxime, ceftriaxone, ceftiolone, cefmenoxime), but became resistant to first and second generation cephalosporins. Acquired resistance to cephalosporins was shown in a strain of Salmonella typhimurium isolated from a patient during cephalexin therapy, and the mechanism of decreased susceptibility was shown to be due to reduced porin production (Medeiros, 1984).

The outer membrane has also been shown to possess another component that will cause resistance in Gram-negative bacteria, the lipopolysaccharide (LPS). Godfrey, Hateld & Bryan (1984) showed that there was a correlation between the LPS structure and resistance of four β-lactam resistant strains of Ps. aeruginosa, two strains excluding hydrophilic antibiotics (ticarcillin and carbenicillin) and two strains differentiating cepham antibiotics by electrical charge (excluding ceftriaxone and allowing in ceftazidime) where the presence of a positive charge increased the antibiotic efficiency (this was not true for penam derivatives, where low molecular weight dianions were most effective). The LPS has also been shown to interact in the penetration of β-lactams by other mechanisms. Barnickel, Labischinski & Giesbrect (1983) concluded from x-ray diffraction studies of LPS that the highly ordered arrangement of the LPS represented a mechanical barrier to antibiotics, and Legakis & Shearer (1984) demonstrated that the LPS in Ps. aeruginosa was usually associated with the ompF porin and the state of the LPS directly influenced the number of open functional porin channels.

The most important mechanism of resistance to β-lactam antibiotics to date is that afforded by β-lactamase. Enzymes that will destroy cephalosporins or both are classed β-lactamases with the enzyme classification number EC 3.5.2.6. The first report of plasmid mediated resistance in Gram-negative bacteria was published in 1965 (Datta & Kontomichalou, 1965), where resistance to β-lactams was associated with the production of specific β-lactamases. The general properties of β-lactamases are that the enzyme will form a reversible complex with the substrate (β-lactam antibiotic), will open the β-lactam ring and the products of the reaction will be the active enzyme and an open-ring or fragmented non-microbiologically active β-lactam product. β-lactamases from Gram-negative bacteria have been classified by inhibition by certain compounds, iso-electric point, molecular weight, gene expression and by substrate profiles (Richmond & Sykes, 1973; Matthew & Harris, 1976; Sykes & Matthew, 1976). More recently β-lactamases have been classified by their amino acid sequences, leading to three distinct groups of enzymes Class A, B and C (Ambler, 1980; Jaurin & Grundstrom, 1981). Richmond & Sykes group 3 β-lactamases have been shown to be class A enzymes, and Richmond & Sykes group 1, class C enzymes. The production of β-lactamase can be chromosomally or plasmid mediated, and in many cases these bacteria will produce β-lactamases constitutively (i.e. the enzyme can be detected in the absence of a β-lactam), some species will also produce...
inducible \( \beta \)-lactamase. In Gram-negative bacteria it has been shown that \( \beta \)-lactamases are located in the periplasm between the inner membrane and the peptidoglycan, providing another obstacle between the \( \beta \)-lactam and its targets, the penicillin-binding proteins (PBPs). It is thought that \( \beta \)-lactamases may have evolved from penicillin-sensitive enzymes, that is the PBPs, involved in peptidoglycan biosynthesis (Tipper & Strominger, 1965; Pollock, 1967) and this theory is supported by the finding that several Gram-negative bacterial low molecular weight PBPs possess weak \( \beta \)-lactamase activity. Waxman & Strominger (1980) showed sequence homology between the active site of D-alanine carboxypeptidase from Bacillus spp. and the active-site serine of class A \( \beta \)-lactamases. This work was extended and sequence homology demonstrated between E. coli PBPs 5 and 6 to the D-alanine carboxypeptidases from Bacillus spp. and class A \( \beta \)-lactamases (Waxman, Amanama & Strominger, 1982). It has been shown that these two groups of enzymes possess similar catalytic mechanisms.

The production of third generation cephalosporins has recently led to a new aspect of resistance due to \( \beta \)-lactamase production. Richmond & Sykes type 1 enzymes are usually inducible and production of the enzyme is under 'repressor' control, therefore the concentration of \( \beta \)-lactamase is low until the exposure of the bacteria to certain \( \beta \)-lactams. The \( \beta \)-lactam causes the repressor to be released therefore the gene coding for \( \beta \)-lactamase can be transcribed and \( \beta \)-lactamase is produced copiously; removal of the \( \beta \)-lactam will allow the repressor to exert its control again and the concentration of \( \beta \)-lactamase will become low as transcription of the gene coding for the enzyme production is blocked. In one in \( 10^7 \) bacteria a mutation may occur where the repressor is altered in such a way that transcription can occur freely, and \( \beta \)-lactamase is now produced constitutively at high levels in these mutants and their progeny. New cephalosporins such as cefoxitin or ceftazidime allow the rapid multiplication of derepressed mutants by inhibiting the 'normal' inducible population; although normally resistant to \( \beta \)-lactamase hydrolysis, these compounds are not effective against the high levels of enzyme produced in the derepressed mutants thereby providing a selective environment with a population of bacteria resistant to most \( \beta \)-lactam antibiotics. \( \beta \)-Lactams not affected by these enzymes are compounds that bind specifically to PBP 2 in E. coli (Sanders & Sanders, 1983). The high concentrations of enzymes produced in the derepressed mutants has led to controversial theories on the mechanisms of resistance of these enzymes to the so-called \( \beta \)-lactam stable antibiotics. One theory is that these enzymes will bind to the \( \beta \)-lactam in the usual enzymic manner but due to the chemical side groups or alterations in the \( \beta \)-lactam ring are unable to hydrolyse the molecule and will therefore 'trap' the \( \beta \)-lactam in a non-hydrolysable manner (Then & Anghern, 1982; Olson et al., 1983). However, there are drawbacks to this theory and there is evidence that these compounds are hydrolysed, but due to the insensitivity of the assay procedure used by most workers it has been undetectable (overwhelming concentrations of substrate compared to the low concentrations of enzyme used in the assay), the rate of hydrolysis however may be slower than in conventional enzymic hydrolysis of first generation cephalosporins (Piddock, unpublished data; N.A.C. Curtis & D. Livermore, personal communications). Examples of therapy failures due to the selection of mutants with derepressed \( \beta \)-lactamases were seldom seen prior to the clinical use of third generation cephalosporins, but have since been shown in strains of Enterobacter cloacae, Pseudomonas aeruginosa, Serratia spp., Providencia spp., Acinetobacter spp., Citrobacter spp. and occasionally with species of E. coli and Klebsiella spp. The compounds commonly involved are cefoxitin, latamoxef (moxalactam), cefamandole, ceftriaxone, and ceftazidime (Preheim et al., 1982; Beckwith & Jahre, 1980; Sanders et al., 1982).

Once the \( \beta \)-lactam antibiotic has traversed the periplasm it only has to bind to its target site (the PBPs) to exert its bactericidal action. However recent work, predominantly in Gram-negative species, has shown that even PBPs may become altered, thereby causing decreased susceptibility to \( \beta \)-lactams. Laboratory mutants with resistance to mecillinam due to PBP alterations was first shown by Spratt (1977), and a retrospective study of mecillinam/resistant E. coli strains showed the mechanism to be similar. These mutants were made with a PBP that was temperature-sensitive, so at certain temperatures (>24°C) the PBP was altered and could not be detected in the PBP assay, these mutants were also very slow growing at 30°C compared to the parent; the clinical isolates that were resistant to mecillinam were also slow growing and had no detectable PBP 2, and mecillinam was not lethal. It is thought that the slow growth rate allowed cell division and therefore viability.

In 1980 there were several reports of
resistance in bacteria attributed to PBP alterations. Dougherty, Koller & Tomasz (1980) examined the PBPs in β-lactamase-negative ampicillin-resistant Neisseria gonorrhoeae. The 20 strains examined had come from different geographical regions and the PBPs were examined in envelope preparations and in growing cells; five of the isolates which were resistant to penicillin G had reduced affinity for two of the PBPs compared with a penicillin-sensitive strain. Isogenic mutants were made and the results confirmed the finding in the clinical isolates, that is that PBP 1 and PBP 2 in N. gonorrhoeae show decreased binding to penicillin G in resistant strains. Perchsen & Bryan (1980) examined Streptococcus pneumoniae and found that resistance could be associated with altered PBPs. PBP 1, 2 and 3 were not detected so readily and an extra band appeared described as PBP 2. It has also been shown in South African strains of Str. pneumoniae that penicillin resistance involves sequential and cumulative alterations in a number of penicillin-sensitive enzymes, the changes affecting the properties of the PBPs such as the affinity for penicillin G, the quantity and turnover rate of the enzyme or the appearance of new or modified enzymes (Zighelboim & Tomasz, 1981). The PBPs of cloxacillin-resistant Bacillus subtilis strains have been shown to have an altered PBP 2a (Klepe, Yu & Strominger, 1982) and laboratory synthesized group A streptococcus mutants that tolerate penicillin have lower detectable concentrations of PBP 3 and penicillin-resistant mutants had altered PBP 3 (Gutman & Tomasz, 1982). Rodriguez-Tebar et al. (1982) attributed the decreased susceptibility of some Pseudomonas spp. to β-lactams, to a low affinity of Pseudomonas spp. PBPs, and correlated carbencillin resistance with decreased PBP affinity in some instances. Much work has been done examining the PBPs of methicillin-resistant Staph. aureus and several workers have attributed this resistance with PBP changes (Brown & Reynolds, 1980; Hayes et al., 1981; Georgopapadakou, Smith & Bonner, 1982; Utsui et al., 1983; Brown & Reynolds, 1983; Hartman & Tomasz, 1984). Brown & Reynolds (1980) demonstrated the appearance of a new or modified PBP with low affinity to β-lactams which was implicated in resistance, and Hayes et al. (1981) showed an alteration in the affinity of PBP 3 in a resistant clinical isolate. Two groups of workers Utsui et al. (1983) and Hartmann & Tomasz (1984) have demonstrated the presence of an altered PBP 2 with a new PBP, PBP 2, at 78,000 daltons which is common in intrinsically resistant staphylococci, confirming the earlier observations of Brown & Reynolds in 1980. Georgopapadakou et al. (1982) examined resistance in Staph. aureus to oral cephalosporins and associated it with two PBPs, PBP 2 was increased in amount and had a satellite band, and PBP 3 was either not detected or substantially reduced. The most recent report of PBP alterations causing resistance was in Haemophilus influenzae, where different PBP profiles and PBP affinities, for β-lactamase-negative ampicillin-resistant strains were obtained when compared to sensitive strains (Parr & Bryan, 1984; Mendelman et al., 1984). It can be seen that alteration in PBPs as a mechanism of resistance has to date been found primarily in Gram-positive clinical isolates with the major mechanism being the decreased binding of the PBP to the β-lactam antibiotic. This may be due to more selective pressure on Gram-positive bacteria than on Gram-negative bacteria, as the envelope in Gram-negative bacteria is far more efficient a barrier to the environment than the Gram-positive envelope and offers many alternative mechanisms of resistance that do not affect proteins with such important metabolic roles as PBPs.

In reality the fine interplay between the various mechanisms of resistance in the Gram-negative bacteria is reflected in the emergence of resistance to a new antibiotic. An ideal β-lactam antibiotic would (at present) penetrate the external polysaccharides and outer membrane efficiently, not be destroyed (by any mechanism) by β-lactamases, not induce β-lactamase production and bind PBPs with such extreme affinity, that even if a PBP alteration emerges increasing the MIC ten-fold the bactericidal concentration will still be within therapeutically achievable levels.

L. J. V. PIDDOCK
R. WISE
Department of Medical Microbiology, Dudley Road Hospital, Birmingham

References


Harder, K., Nikaido, H. & Matsuhashi, M. (1981). Mutants of *E. coli* that are resistant to certain \( \beta \)-lactam compounds lack the OmpF porin. *Antimicrobial Agents and Chemotherapy* 20, 549-52.


Leading articles


WIN 51711, a new systematically active broad-spectrum antipicornavirus agent
Picornaviruses are a major cause of viral-associated morbidity in humans, and can result in death in immunocompromised patients and neonates. The Picornaviridae family consists of two groups of viruses associated with human disease: the rhinoviruses and enteroviruses. The rhinovirus group consists of over 120 serotypes which are the causative agents of one-third to one-half of all upper respiratory illnesses, normally referred to as the 'common cold' (Couch, 1984). It is estimated that each individual, on the average, experiences from two to five colds per year (Fox & Hall, 1980), resulting in approximately 250 million workdays of restricted activity per year in the US alone (Merigan, 1982).

The enterovirus group consists of at least 68 different viral serotypes, 63 of which are included in the following four subgroups: poliovirus (3 serotypes), Coxsackie A viruses (23 serotypes), Coxsackie B viruses (6 serotypes) and echoviruses (31 serotypes). Five additional recently isolated serotypes have not been placed in any of the four established subgroups due to changes in nomenclature.

Based on data from the Virus Watch Program, it is estimated that 5–15 million enteroviral infections occur each year in the US (Kogon et al., 1969), with only half of these infections resulting in symptomatic illness (Spigland et al., 1966). Clinical enteroviral syndromes range from mild upper respiratory disease with or without myalgia and fever, summer exanthems, herpangina and acute hemorrhagic conjunctivitis, to aseptic meningitis, myocardiitis/pericarditis, polymyelitis, encephalitis, infectious hepatitis and neonatal sepsis. The type of syndrome seen is largely dependent on the infecting serotype. The more serious syndromes such as polymyelitis and aseptic meningitis are associated with only a few enterovirus serotypes, while mild upper respiratory disease and other less severe syndromes are associated with all serotypes.

At present, there is no specific treatment for picornavirus infections, although in excess of $1 billion is spent each year on palliative 'cold' remedies (Couch, 1984). In recent years, the clinical usefulness of a number of synthetic agents (enviroxime, dichloroflavan, Ro-09-0410) has been assessed in volunteers experimentally infected with rhinovirus (Hayden & Gwaltney, 1982; Phillpotts et al., 1983; Phillpotts et al., 1984). However, none of these compounds have demonstrated efficacy with the formulations and routes tested.

Recently, the synthesis of WIN 51711, a novel antiviral agent possessing activity in vitro against both enteroviruses and rhinoviruses, was reported (Diana et al., 1984):

![WIN 51711](https://example.com/w51711.png)

WIN 51711 has been shown to exert its antiviral effect through a direct interaction with picornaviral capsid protein(s) (Fox et al., 1984). Mechanism studies have demonstrated that the reversible drug-virus interaction 'stabilizes'...